postulado de FRANZEN (1956) sustenta que existe una relación entre la morfología del espermatozoide y la biología de la fecundación, y que la forma de las células está influenciada no sólo por las relaciones filogenéticas, sino también por los requerimientos del espermatozoide en el proceso de fecundación. (AFZELIUS, 1979).

De lo expuesto cabe inferir que el espermatozoide de *Sarasinula linguaeformis* ostentaría un modelo de tipo primitivo, a pesar que su modo de fecundación se realiza a través de órganos copuladores. Este hecho podría indicar que la morfología del espermatozoide aquí descrito, estaría vinculado más bien con las relaciones filéticas que con la biología de la fecundación, acorde a la posición primitiva de los Veronicellidae dentro del contexto de los Euthyneura. Esto último se fundamentaría porque en los Opisthobranchia y Pulmonata, la forma de los espermatozoides se modifica y no se encontraron indicios de la condición primitiva en ninguno de los casos estudiados (FRANZEN, 1955).

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Presence of abnormal cilia in the mucosa of the male gonoduct of *Bolinus (Murex) brandaris* (Gastropoda, Prosobranchia, Muricidae)

Presencia de cilios anormales en la mucosa del gonoducto masculino de *Bolinus (Murex) brandaris* (Gastropoda, Prosobranchia, Muricidae)

María José AMOR*

ABSTRACT

Abnormal cilia, in which the bridges between the plasma membrane and the external microtubules are broken, are found in the mucosa of the male gonoduct of *Bolinus (Murex) brandaris*. As some authors have described this kind of cilia in other species as artifacts produced by the osmolarity of the fixative medium, we fixed the samples in different osmolarity media, finding in all cases the presence of these abnormal cilia. A description of the ultrastructure of the abnormal cilia of the male gonoduct of *Bolinus (Murex) brandaris* and suggestions concerning the role of the paddle cilia are presented.

RESUMEN

En la mucosa del gonoducto masculino de *Bolinus (Murex) brandaris* han sido detectados cilios anormales, caracterizados por la ruptura de los puentes que unen los microtúbulos externos del axonema y la membrana plasmática. Como algunos autores han descrito en otras especies esta clase de cilios como artefactos producidos por la osmolaridad del medio de fijación empleado, hemos fijado muestras con diferentes osmolaridades obteniendo siempre la presencia de estos cilios anormales. Una descripción de la ultrastructura de los cilios anormales del conducto deferente de *Bolinus (Murex) brandaris*, así como diferentes sugerencias sobre su posible misión, son discutidas en el presente trabajo.

PALABRAS CLAVE: Cilios anormales, medio de fijación, gonoducto masculino, *Bolinus (Murex) brandaris*, Gastropoda, Muricidae.
KEY WORDS: Abnormal cilia, fixative medium, male gonoduct, *Bolinus (Murex) brandaris*, Gastropoda, Muricidae.

INTRODUCTION

Swellings in the plasma membrane of some cilia were first described by Luther 85 years ago (WASIK AND MIKOLAJCZYK, 1991). As the shape of cilia may be changed because of these swellings, TAMARIN, LEWIS AND ASKEY (1976), called them paddle cilia, while HEIMLER (1978) named them discocilia. Nevertheless, other authors refer to them indistinctly as paddle cilia or discocilia (DILLY, 1977a, b; MATERA AND DAVIS, 1982 and WASIK AND MIKOLAJCZYK, 1991).

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They are frequently seen in marine invertebrates, and several authors have assigned various roles to them such as transport (Dilly, 1977a, b; Wasik and Mikolajczyk, 1991), a chemosensitive fuction (Matera and Davis, 1982), locomotion (Heimler, 1978) and even related them to parasitism (Durfort, Bozzo, Poquet, Sagrista, García Valero, Amor and Ribes, 1990). They seem not to be present in vertebrates, although swellings in membrane of cilia have been described in the olfactory epithelium of frogs and mice, and in dendrites of the crab *Pagurus hirsutiusculus*, (Wasik and Mikolajczyk, 1991). Other authors suggest they are artifacts produced by the difference in osmolarity of the fixative medium and the sea water (Ehlers and Ehlers, 1978; Short and Tamm, 1991), although Matera and Davis (1982) have observed them *in vivo* in light microscopy using isotonic medium with sea water in the ciliated *Cymatocilis convallaria*, and Heimler (1978) detected them *in vivo* with an interference microscope.

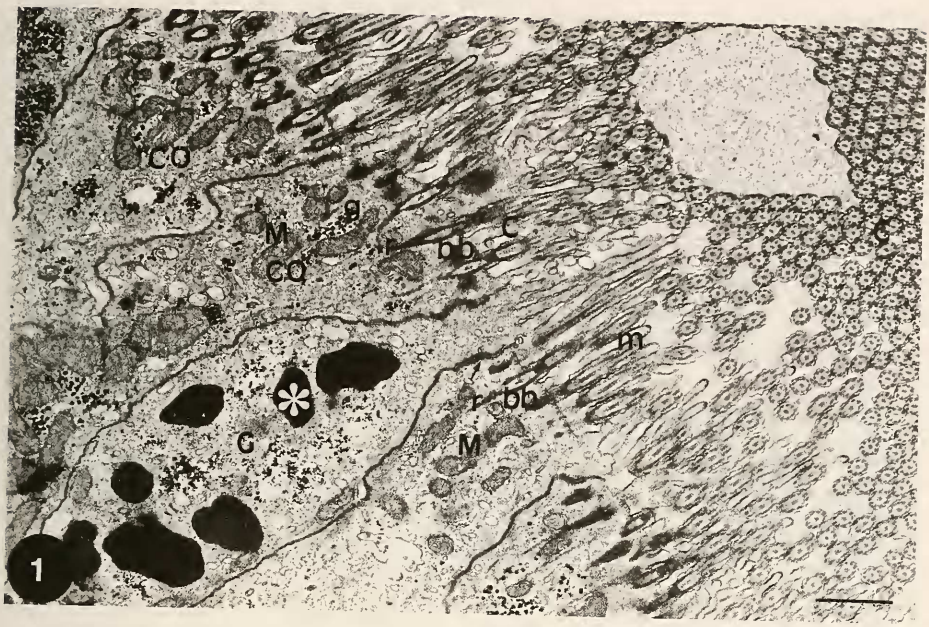
The presence of abnormal cilia in the male gonoduct of *Bolinus (Murex) brandaris* fixed in different osmolarity media and its possible evacuation role are discussed in this paper.

MATERIAL AND METHODS

Specimens of *Bolinus (Murex) brandaris* were collected off the Mediterranean coast (St. Carles de la Ràpita, Tarragona). Deferent ducts and penis were carefully removed and slices of about 300 Å were processed for transmission

(Right page). Figure 1. Panoramic view of the mucosa of the gonoduct of *Bolinus brandaris*, with columnar cells (CO) showing cilia (C) sectioned at different levels, microvilli (m) mitochondria (M) and abundant glycogen granules (g). A goblet cell (G) with granules at different degrees of maturity (asterisk) is also seen. (bb: basal bodies, C: Cilia, CO Columnar cells, G: goblet cell, g: glycogen granules, M: mitochondria m: microvilli, r: ciliary rootlets). Penis region. Fixed by hypertonic medium. Figure 2. Longitudinal section of a cilium showing the characteristic striated rootlet (r), the basal body (bb), the transition region (T) and the axoneme (a). Notice the bridges between the plasma membrane and the external microtubules at the beginning of the cilia (arrows). Deferent region. Fixed by tannic acid method. Hypotonic medium. Figure 3. Cross section of cilia at different levels showing rootlets (r), basal bodies (bb), transition region (T), cilia (C). Note bridges between the external microtubules and the plasma membrane in the transition region (arrows). Abnormal cilia (star), and *adhaerens* junctions between adjacent cells (asterisk) are also detected. Deferent region. Isotonic medium. Scale bars 1 μm.

(Página derecha). Figura 1. Panorámica del epitelio de la mucosa del gonoducto masculino de Bolinus brandaris *en la región del pene, fijado en medio hipertónico, mostrando las células prismáticas (CO), con los cilios seccionados a diferentes niveles (C), microvilli (m), mitocondrias (M), así como abundantes gránulos de glucógeno (g). Puede observarse también la presencia de una célula caliciforme (G) con gránulos en diferentes grados de maduración en su interior (asterisco). (bb: corpúsculos basales; C: cilios; CO: células prismáticas; G: célula caliciforme; g: gránulos de glucógeno; M: mitocondrias; m: microvilli; r: raíces ciliares). Figura 2. Corte longitudinal de un cilio morfológicamente normal del conducto deferente mostrando la característica estriación de la raíz ciliar (r), el corpúsculo basal (bb), la región intermedia (T) y el axonema (a). Pueden observarse asimismo los puentes entre la membrana plasmática y los microtúbulos externos en la zona del nacimiento del cilio (flechas). Fijación con ácido tánico en medio hipotónico. Figura 3. Corte transversal de cilios del conducto deferente a diferentes niveles mostrando las raíces ciliares (r), los corpúsculos basales (bb), la región intermedia (T) y los axonemas (a). Obsérvense los puentes en la región intermedia entre los microtúbulos externos y la membrana plasmática (flechas). Se observa también la presencia de cilios anormales (estrella), así como algunas uniones* adhaerens *entre células vecinas (asterisco). Medio isotónico. Escalas 1 μm.*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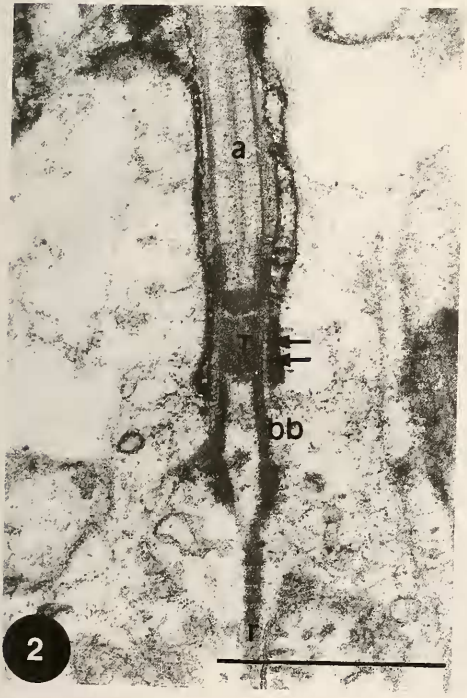
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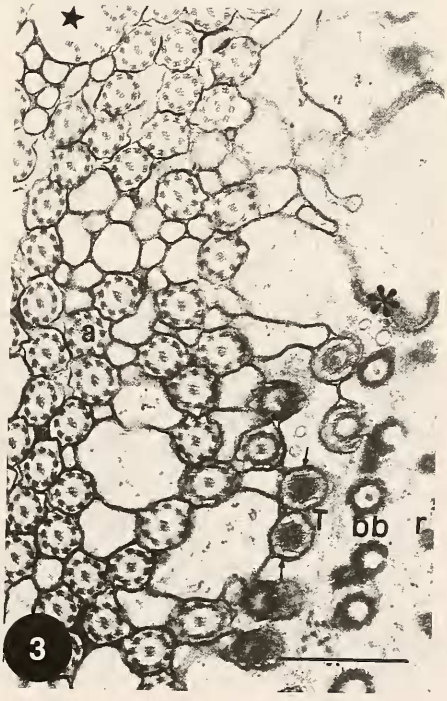
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300 Å were processed for transmission electron microscopy.

As we wanted to detect the effects of the osmolarity of the fixation medium on the shape of the cilia in the mucosa, three kinds of fixation media were prepared:

-Hypotonic medium: Conventional medium consisting of 2.5% glutaraldehyde, 3.5% paraformaldehyde, buffered with cacodylate buffer (pH 7.2-7.4). Osmolarity: 454 mOsmols.

-Isotonic medium: To the conventional medium, we added NaCl progressively until obtaining an isotonic medium with sea water (920 mOsmols, SHORT AND TAMM, 1991).

-Hypertonic medium: We added NaCl to an osmolarity of 1279 mOsmols.

To visualize the ciliary bridges between the plasma membrane and external microtubules, 1% tannic acid was added to the glutaraldehyde in some samples, as indicated by TORIKATA (1988).

After washing with the same buffer, all samples were postfixed with 1-2% OsO_4 and again washed with the same buffer.

The samples were dehydrated and embedded in Spurr's resin (SPURR, 1969). Before ultrathin sections were cut, 1μm sections were prepared and stained with 1% methylene blue-borax to select appropiate areas for transmission electron microscopy observations. The ultrathin sections (about 300 Å) were contrasted with uranyl acetate and lead citrate (REYNOLDS, 1963).

For scanning electron microscopy observations, some dried samples were passed through amyl acetate and dried to critical point.

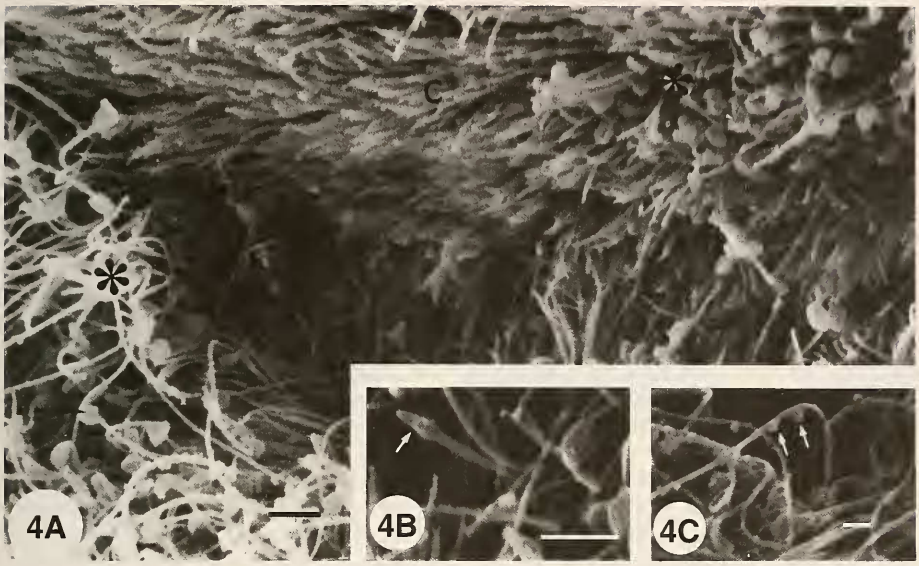
To improve the observation of the dispersion of the microtubules, samples of 0.5 μm were observed in a Hitachi H 800 MT, with an acceleration voltage between 150-200KV, and a number 4 objective diaphragm, although no new information was obtained. The remaining observations were made using Philips EM 200 and Philips EM 301 electron microscopes, and a Hitachi S-2, 300 scanning electron microscope in the Electron Microscope Service at the University of Barcelona.

RESULTS

The mucosa of the gonoduct of *Bolinus (Murex) brandaris* is formed by a columnar epithelium which shows abundant cilia and microvilli, and among these cells some goblet cells are found (AMOR, 1990a, b; AMOR, 1992) (Fig. 1). Cilia are longer in the penis region than in the deferent region, and are set in the characteristic basal bodies that continue in the typical striated rootlets, longer in the first part of the deferent duct than in

(Right page). Figure 4. Abnormal cilia observed by scanning microscopy. Penis region hypotonic medium. A: panoramic view (C: normal cilia; asterisk: discocilia); B: detail of an abnormal cilium with a subapical swelling (arrow); C: detail of abnormal cilia with swellings in their central region of the cilia (arrows). Figure 5. Panoramic view of abnormal cilia in transmission electron microscopy. Notice the presence of disordered axonemes (arrowheads) as well as double axonemes (arrows), within the swellings of the plasma membrane (asterisk). Normal cilia (C) are also seen. Penis region. Hypotonic medium. Scale bars 1 µm.

(Página derecha). Figura 4. Cilios anormales observados con microscopía electrónica de barrido. Región del pene. Medio hipotónico. A: vista panorámica (C: cilios normales. Asterisco: discocilios); B: detalle de un cilio anormal con una dilatación subapical (flecha); C: detalle de cilios anormales con dilataciones en la región media (flechas). Figura 5. Vista panorámica de cilios normales (C) y cilios anormales con microscopía electrónica de transmisión (región del pene), pudiendo observarse la desorganización de los axonemas (puntas de flecha) y la presencia de dos secciones de axonema (flechas) dentro de las dilataciones de la membrana plasmática (asterisco). Medio hipotónico. Escalas 1 µm.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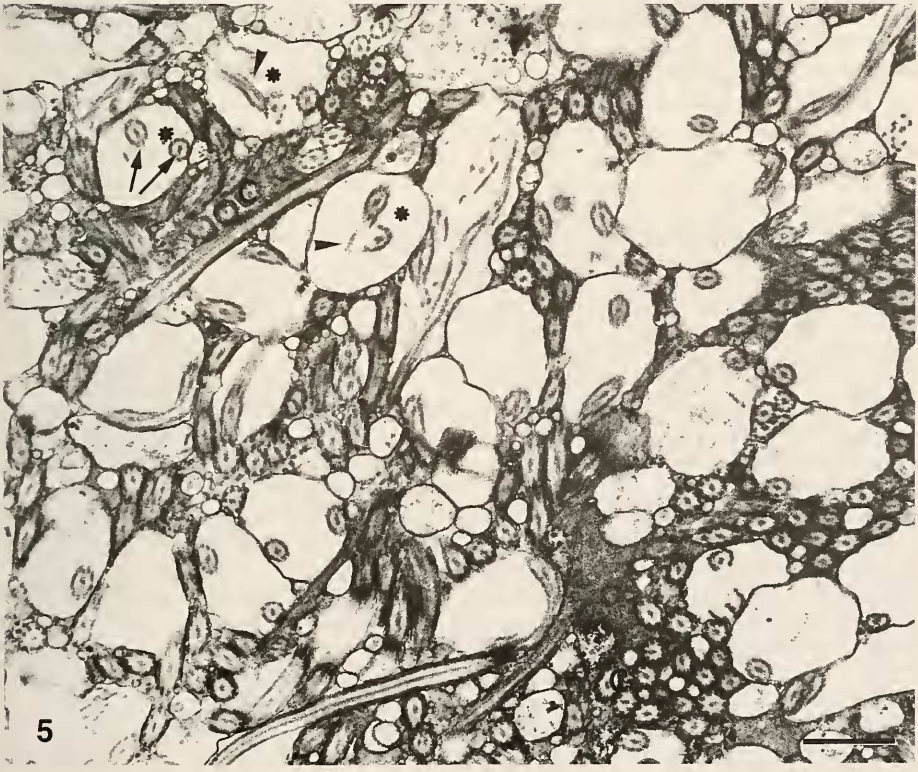
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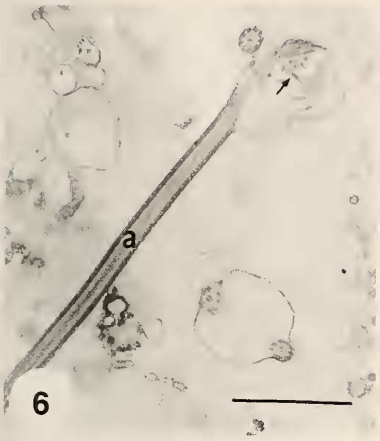
the rest of the gonoduct. This seems to be related with the higher density of the semen at the first levels of the gonoduct, where these smaller cilia and longer rootlets could give more strength to evacuate it, as we have pointed out previously (AMOR, 1991).

At the transition region, cilia show the characteristic champagne-glass shaped (necklace) bridges (Fig. 2) described by DENTLER (1981). These bridges, 5-6 in number, are 36.6 nm long and 6.1 nm thick, with a periodicity of 18.3 nm. In the rest of the cilium, the bridges between the plasma membrane and the external microtubules described by DENTLER (1990) are seen. These bridges are 17 nm thick and 13.6 nm in length, with a periodicity of 40 nm (Figs. 2, 3).

These bridges are sometimes broken and swollen formations 1 μm wide appear in the ciliary membrane (AMOR, 1992) (Figs. 3, 5-11, 13 and 14). If these swellings are found in the central region of the cilium, only some bridges of the axoneme are broken, and some parts of the axoneme are still connected to the plasma membrane. However, if all bridges are broken, the axoneme appears free and separated from the plasma membrane. In both cases, the axoneme remains unaltered (Fig. 8 and 14B). In contrast, if these swellings occur at the tip or near the tip of the cilium, the axoneme then curves, and may form one or several loops inside the swelling of the membrane. Connections between doblets of microtubules are often broken, and the axonemes become completely disordered (Figs. 4-13 and 14A). In our material we have detected 23% of swellings in the central region of the

(Right page). Figure 6. Longitudinal section of a discocilium at the deferent duct level. Notice the swelling of the plasma membrane as well as the disordering of the axoneme at the top of the cilium (arrow), while the rest of the axoneme remains unaltered. Isotonic medium. Figure 7. Different arrangements of the axoneme in the swellings of the plasma membrane in the central region of the cilia. Glycogen granules (g) are also seen. Penis level. Tannic acid technique. Hypotonic medium. Figure 8. Detail of a longitudinal section of a swelling of the plasma membrane in the central region of a cilium (star). In this case, the axoneme (a) remains unaltered. Deferent region. Hypotonic medium. Figures 9-12. Cross sections of abnormal cilia showing different arrangements of the axoneme. Figure 9 shows the loops of the axoneme: «a» and «c» have the same orientation of the peripheric microtubules while «b» shows peripheric microtubules in the opposite direction (arrow). Deferent duct level. Hypertonic medium. Figure 13. Panoramic view of abnormal cilia (asterisks) showing microtubules undergoing dispersion (arrows). Besides, normal cilia are seen (C) as well as some glycogen granules (g). Hypotonic medium. Deferent level. Scale bars 1 µm.

(Página derecha). Figura 6. Sección longitudinal de un discocilio del conducto deferente mostrando la dilatación de la membrana plasmática así como la desorganización del axonema en el extremo del mismo (flecha), mientras el resto del axonema permanece intacto. Medio isotónico. Figura 7. Diferentes disposiciones adoptadas por el axonema en las dilataciones a nivel medio, en un corte del gonoducto a nivel del pene. También se observan algunos gránulos de glucógeno (g). Medio hipotónico. Acido tánico. Figura 8. Corte longitudinal de un cilio del conducto deferente presentando una dilatación a nivel de la región media (estrella). Obsérvese como en este caso, el axonema (a) no está alterado. Medio hipotónico. Figuras 9-12. Secciones transversales de varios cilios anormales mostrando las distintas disposiciones adoptadas por el axonema. En la Figura 9, pueden observarse las curvaturas del mismo: «a» y «c» presentan la misma orientación de los microtúbulos periféricos mientras «b» presenta los microtúbulos periféricos orientados en sentido opuesto (flechas). Conducto deferente. Medio hipertónico. Figura 13. Imagen panorámica de cilios anormales (asteriscos) mostrando los microtúbulos del axonema en dispersión (flecha). A su lado se aprecian algunos cilios morfológicamente normales (C), así como algunos gránulos de glucógeno (g). Conducto deferente. Medio hipotónico. Escalas 1 µm.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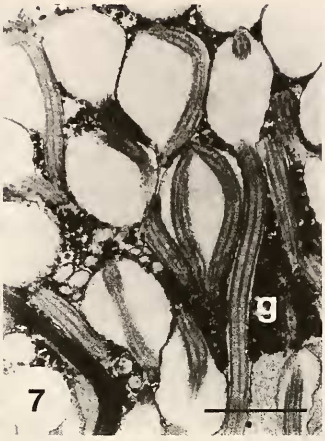
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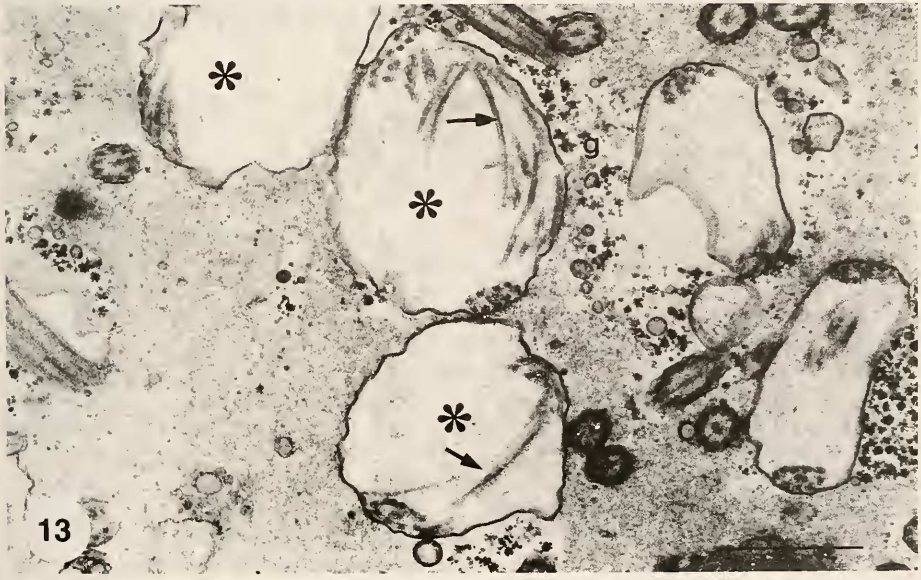
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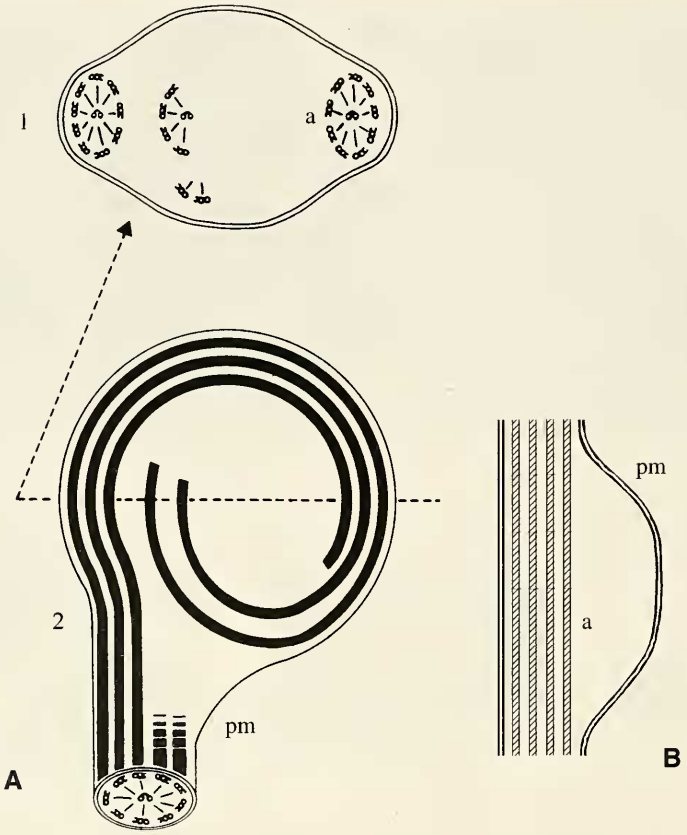

Figure 14. Schematic representation of the arrangement of microtubules in the abnormal cilia. A: swellings located in the tip of the cilium; 1: cross section, 2: longitudinal section. B: swellings located in the central region of the cilium; a: axoneme, pm: plasma membrane.
Figura 14. Esquema de la disposición de los microtúbulos en los cilios anormales. A: dilataciones situadas en el extremo del cilio; 1: sección transversal, 2: sección longitudinal. B: dilataciones situadas en la región media del cilio; a: axonema, pm: membrana plasmática.

plasma membrane, and 65.7% of swellings in the tip of the cilia. In the later case, a 59.2% could have two or more loops with an axoneme disorder, and only 6.5% do not show any axoneme disorder. The rest of the ciliary shaft remains identical to normal cilia.

These abnormal cilia seem not to be distributed uniformly: there are areas where abnormal cilia are more abundant (Fig. 4A), and they represent about 4-6% of the total cilia. Different buffers as well as different osmolarities of the fixation dium have no effect on the proportion or configuration of these peculiar formations.

DISCUSSION

Along the gonoduct of *Bolinus (Murex) brandaris* structure, there are two populations of cilia: normal cilia with the characteristic configuration of rootlets, basal bodies and the 9+2 configuration of the axoneme (Amor, 1990a, b; Amor, 1992) and a 4-6% of abnormal